TISSUE CULTURE

Methods and Applications

Edited by

PAUL F. KRUSE, JR., and M. K. PATTERSON, JR.

Biomedical Division
The Samuel Roberts Noble Foundation, Inc.
Ardmore, Oklahoma

ACADEMIC PRESS New York San Francisco London 1973

A Subsidiary of Harcourt Brace Jovanovich, Publishers

CHAPTER 15

Karyology of Cells in Culture

A. Preparation and Analysis of Karyotypes and Idiograms

T. C. Hsu

The chromosome constitution of cells *in vitro* has been one of the most useful criteria for monitoring cell populations. In a number of cases, contamination of one cell culture with another was detected by karyological analysis. When the chromosomal features of the two cell cultures are very different, even casual microscopic inspection will suffice.

It is well known that cell cultures *in vitro* frequently change their chromosomal constitutions. Karyotypical analysis is useful for monitoring the "normalcy" of the cell populations under consideration.

There are many procedures for karyological preparations, each slightly different from others in some minor details. For details one may find the book edited by Yunis[1] useful. The procedure described below is probably the easiest to follow, since not much "art" is involved.

Procedure

1. Use cell cultures in the active (exponential) growth phase, preferably feeding them the day before scheduled harvest.
2. Add Colcemid or Velban (final concentration of 0.06 μg/ml) to the culture and incubate for 2 hours.
3. Decant medium, rinse the monolayer culture with physiological saline, and dislodge the cells with a Pronase solution (Pronase 0.01% dissolved in physiological saline). Trypsin solution can be used in lieu of the pronase solution. For suspension cultures, this step is omitted.
4. As soon as the monolayer cells are dislodged, they should be centrifuged to pack into a pellet. The speed of centrifugation is immaterial as long as the cells can be pelletted within a few minutes. Decant the Pronase or trypsin solution, and introduce a hypotonic solution (one part of growth medium and two parts of distilled water).
5. Suspend the cells in the hypotonic solution (5–7 ml) with a Pasteur pipette, leave for 5 to 10 minutes, and centrifuge.
6. Decant the hypotonic solution and introduce a fixative (three parts methyl

[1] J. J. Yunis, "Human Chromosome Methodology." Academic Press, New York, 1965.

alcohol and one part glacial acetic acid, freshly prepared, 5 ml to each sample) to the centrifuge tube without disturbing the pellet for at least 20 minutes.

7. Suspend cells with a Pasteur pipette; recentrifuge.

8. Decant the old fixative, add fresh fixative, resuspend the cells, and recentrifuge the cells. Repeat this process three times. Finally, reduce the fixative to 1 ml or less, and suspend the cells.

9. Dip alcohol-cleaned slides in a mixture (ice cold) containing 40% methanol and 60% distilled water. Pull out the slide and drain off the excess amount of fluid but do not shake it off.

10. Hold the slide at a 45° angle toward the floor. Place a drop of the final cell suspension on the upper portion of the slide and let the cell suspension spread downward. If the cell suspension is too thin, place more drops on each slide. Dry the slides in air.

11. Stain with acetic orcein (2% orcein in 45% acetic acid, 3–5 minutes) or Giemsa (10% commercial Giemsa stock solution in phosphate buffer, pH 7, 10–15 minutes). Rinse, air-dry, and mount in any conventional mounting medium.

Karyotyping

In all cytological preparations of animal cells, regardless of the method used, there is a certain proportion of ruptured cells. When one tries to determine the diploid number (or the number of a particular specimen), metaphases losing or gaining one or more chromosomes as artifacts are not formidable obstacles since counts of 20 to 30 cells would invariably reveal the predominant number. Furthermore, there would be no particular chromosome or chromosomes involved in the loss or gain. The modal number is the real number. However, in tissues or individuals with chromosome mosaicism, two or more modal numbers may be recorded. Although the two chromosome numbers are not always represented by equal number of metaphases, the second number is usually significantly higher in the distribution curve than the remaining artifacts. Further, karyotype analysis should reveal that the extra chromosome or the missing chromosome invariably involves the same element. Table I gives some examples of data collection with several cases taken from preparations made from human lymphocyte cultures.

In heteroploid cell populations such as long-term cell lines (HeLa, L, etc.)

TABLE I

Chromosome Counts of Four Samples of Human Lymphocyte Cultures

Subject	Chromosome number							Number of cells
	43	44	45	46	47	48	49	
Normal volunteer	1	—	2	28	1	—	—	32
Down's syndrome	—	1	—	2	25	1	—	29
Normal/Down's syndrome mosaic	—	1	1	32	12	—	1	47
Turner's syndrome	—	1	34	1	—	—	—	36

where cells with many different chromosome numbers may exist, the elimination of artifacts becomes a difficult task. Karyotype analysis will not be of great help either, because cells with the same number of chromosomes do not always have the same chromosomes.

Contemporary cytologists use cut-out photomicrographs of metaphase chromosomes for constructing karyotypes. This practice is preferred over camera lucida drawings because photographs greatly reduce the subjectivity in the part of the investigators, especially inexperienced investigators.

In conventional preparations, the metaphase chromosomes exhibit rather limited features for differentiation and recognition: length, position of the centromere (thereby arm ratio), and the presence of secondary constrictions which may occur in special chromosomes. Therefore, in most mammalian species, chromosome pairs with similar morphology are common. When constructing karyotypes, it is necessary to group the chromosomes of similar length and shape if such grouping is feasible. The best example is the karyotype of man, where seven groups are recognized. Within each group, identification of particular chromosome pairs is uncertain with conventional staining procedures. In the karyotypes of some species, e.g., the chromosomes of the laboratory mice, even grouping is not possible because all chromosomes possess the same morphology and there is no distinct break in the length gradation. Only in a few exceptional species, such as the Indian muntjac and several species of Australian marsupials, every pair of chromosomes can be identified without ambiguity.

Figures 1–3 show two extreme and an intermediate case. In Fig. 1 (a karyotype of an Indian muntjac) there is no problem pairing the elements. In Fig. 2 (a karyotype of the laboratory mouse) it is not possible to identify individual pairs. In Fig. 3 (a karyotype of a striped hyena) grouping according to morphological features is feasible, but identifying pairs within each group is subjective.

Idiogram Construction

An idiogram is an idealized karyotype or a statistically representative karyotype. Therefore no single metaphase, whether it be the actual photograph or length measurements, can be considered as an idiogram. An idiogram must be arrived at by the normalization of many karyotypes. It is a time-consuming process if an idiogram is constructed manually. With the aid of computers, the task can be simplified a great deal.[2-4] However, it is pointless to construct idiograms just for the sake of constructing them. It is worth doing only if the chromosomes of a species are widely used in cytological investigations.

As can be seen, the utility of the conventional karyotype and idiogram analysis has many limitations. It is virtually impossible to determine paracentric

[2] M. Mendelsohn, D. Hungerford, B. Mayall, B. Perry, T. Conway, and J. Prewitt, *Ann. N. Y. Acad. Sci.* **157**, 376 (1969).

[3] C. J. Hilditch and D. Rutovitch, *Ann. N. Y. Acad. Sci.* **157**, 333–364 (1969).

[4] J. W. Butler, M. K. Butler, and A. Stroad, *In* "Data Acquisition and Processing in Biology and Medicine" (K. Enslein, ed.), pp. 261–275, Pergamon Press, New York, 1964.

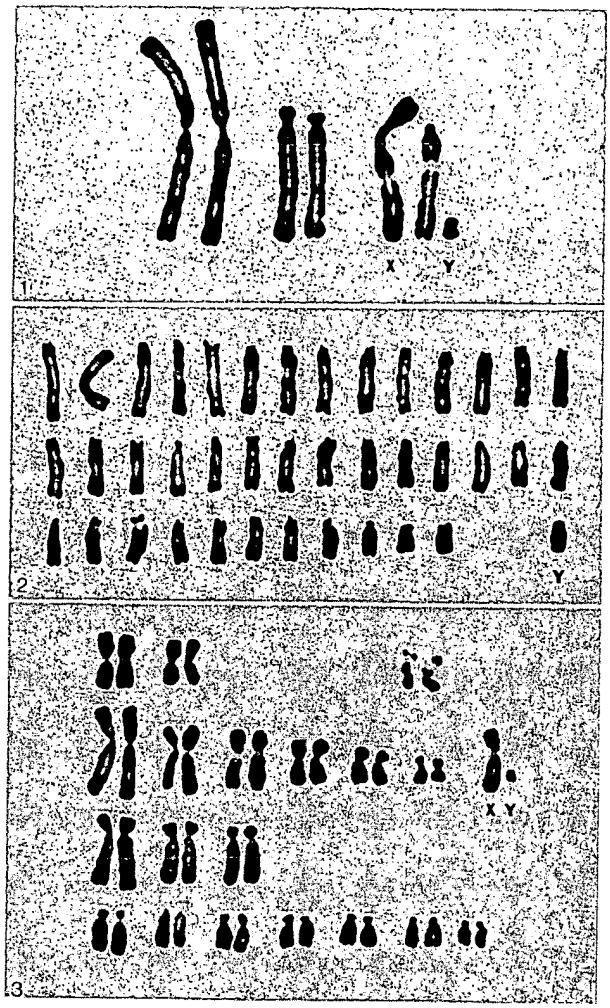

Fig. 1. Karyotype of a male Indian muntjac (*Muntiacus muntjak*), $2n = 7$ (♀ $2n = 6$). A Robertsonian fusion has apparently occurred between the *X* chromosome and the acrocentric autosome, but the homologous autosome was not translocated onto the Y chromosome, thus giving an impression that there are two Y chromosomes. Every chromosome pair of this karyotype is morphologically distinct.

Fig. 2. Karyotype of a male C3H mouse (*Mus musculus*), $2n = 40$. All chromosomes are acrocentric and their lengths form a continuous gradation. Pairing is not possible. The only recognizable elements are the two smallest autosomes and the Y chromosome, which form a group, but identification of the Y is usually arbitrary.

Fig. 3. Karyotype of a male striped hyena (*Hyaena hyaena*), $2n = 40$. The chromosomes can be classified into several major groups, but identification of each pair within a group is equivocal.

inversions or reciprocal translocations of nearly equal portions without examining meiotic behavior. The difficulty has been partially removed by the recent inventions of many new procedures which would reveal characteristic chromosome bands (see next few procedures).